Immunology 1992 **75** 23–28

Effects of *Staphylococcus aureus* cell wall products (teichoic acid, peptidoglycan) and enterotoxin B on immunoglobulin (IgE, IgA, IgG) synthesis and CD23 expression in patients with atopic dermatitis

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Accepted for publication 5 September 1991

SUMMARY

The influence of staphylococcal cell wall products (teichoic acid, peptidoglycan) and enterotoxin B on peripheral blood lymphocytes (PBL) from patients with atopic dermatitis (AD) was investigated. The parameters studied were spontaneous and interleukin-inducible immunoglobulin (IgA, IgE, IgG) synthesis and CD23 expression. PBL from non-atopic donors served as controls. Teichoic acid and peptidoglycan induced an enhanced synthesis of IgA and IgG in normal donors. However, IgA and IgG synthesis in PBL from patients with AD was significantly suppressed by teichoic acid and enterotoxin B. The incubation of PBL from normal donors with enterotoxin B and interleukin-4 (IL-4) or IL-5 led to a significant suppression of IgA and IgG synthesis. Co-stimulation of PBL with teichoic acid or peptidoglycan and IL-4 led to a pronounced increase in IgE synthesis and CD23 expression in patients with AD. Our data indicate that cell wall products and toxins of staphylococci modulate the cytokine-dependent humoral immunity in patients with AD and may be responsible for allergic skin reactions in AD.

INTRODUCTION

Normal as well as diseased skin of patients with atopic dermatitis (AD) is severely colonized with *Staphylococcus aureus*;[1] staphylococci are also prominent in cultures from nasal and pharyngeal sites of these patients.[2] In contrast to normal individuals, *S. aureus* belongs to the resident flora in patients with AD.

The underlying mechanism of this chronic bacterial colonization of the skin as well as the role of *S. aureus* in allergic reactions in AD are still unclear. Differences in sebaceous and sweat secretions have been suggested.[3] Increased adherence of *S. aureus* to epithelial cells obtained from either the skin or nasal mucosa led to the suggestion that either qualitative or quantitative differences in bacterial receptors (e.g. fibronectin) on keratinized cells of AD skin may predispose to the increased carriage of staphylococci.[4] On the other hand, defective host defence mechanisms involved in the control of bacterial infections have also been suggested.[5] Owing to a selective hyporesponsiveness to purified *S. aureus* cell walls in delayed skin reactivity,[6] disorders of T cells in patients with AD have been stated to be responsible for the bacterial colonization. Recently, it has been shown that heat-killed *S. aureus* strongly suppresses IgA and IgG synthesis in PBL from patients with AD *in vitro*.[7] These data indicate that staphylococci are able to modify the humoral immunity of patients with AD in a way which may support the chronic skin colonization.

There is controversy as to whether the chronic colonization of the skin with *S. aureus* in patients with AD is important for the individual course of the disease. In several studies a reduction in the bacterial load by antibiotic therapy has been noted to be associated with clinical improvement.[8] *S. aureus* may also induce a specific immune response in AD patients: increased serum IgE is directed against intact cells, cell wall and soluble antigens of *S. aureus*;[9–11] and basophils of patients with AD respond to *S. aureus* with an IgE-dependent mechanism and release large amounts of histamine.[12] Furthermore, it has been demonstrated that *S. aureus* induces increased IgE synthesis and CD23 expression in patients with AD.[7]

The effects of soluble and defined components of *S. aureus* on host defence mechanisms and allergic reactions in patients with AD are presently unknown.

It was the purpose of this study to investigate the role of the main staphylococcal cell wall products (teichoic acid, peptidoglycan) and enterotoxin B alone and combined in the presence of IL-4 and IL-5 on the immunoglobulin synthesis and CD23 expression of peripheral blood lymphocytes from patients with AD.

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MATERIALS AND METHODS

Isolation of lymphocytes and cell separation

Lymphocytes were obtained from healthy volunteers and from patients with atopic dermatitis (AD). The AD diagnosis was performed according to the criteria of Hanifin and Rajka;[13] the following four basic features were present: a chronic or chronically relapsing dermatitis; flexural lichenification; pruritus; and a personal or family history of atopy (asthma, rhinitis, AD). The serum IgE levels were above 500 ng/ml. The donors did not receive any steroid treatment. Normal students with no personal history of allergic diseases and serum IgE levels below 300 ng/ml served as controls.

Isolation was performed by centrifugation on a Ficoll–sodium metrizoate (Sigma Chemical Co., Munich, Germany) gradient according to Böyum.[14] Briefly, heparinized venous blood (200 ml) was layered over Ficoll–sodium metrizoate (density = 1075 g/ml) and centrifuged at 375 ***g*** for 45 min. Cells at the interface above the Ficoll–metrizoate were removed and washed three times with RPMI-1640. These cells are referred to as 'peripheral blood lymphocytes' (PBL).

Culture conditions

The basic culture medium was RPMI-1640 supplemented with 2 mM glutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin, 10 mM HEPES and 20 mM sodium hydrogen carbonate. Medium containing 10% foetal calf serum (FCS) is referred to as RPMI-1640 with 10% FCS.

Cell suspensions containing 1×10^6/ml viable cells in RPMI-1640 and 10% FCS were dispensed into each well of 6- and 24-well plates. Stimuli were added and the cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 in air.

The IgA, IgE and IgG contents of culture supernatants were determined by radioimmunoassays.

Lymphokines

Freshly prepared PBL were suspended in culture medium with purified recombinant human IL-4 (quantity used: 20 U/ml) and IL-5 (quantity used: 10 U/ml). IL-4 was obtained from Genzyme (purchased from IC Chemikalien, Munich, Germany) and IL-5 from Amersham (Braunschweig, Germany).

Bacterial products

Purified teichoic acid, peptidoglycan and enterotoxin B from *S. aureus* were obtained from Sigma. The substances were protein A-free as determined by immunoblot technique. As positive controls a *S. aureus* strain and purified protein A (Sigma) were used. The detection was performed with a mouse IgG2a antibody (data not shown).

Radioimmunoassay for IgA, IgE and IgG

The IgA, IgE and IgG contents of culture supernatants were determined by a solid-phase radioimmunossay (RIA) on Day 10. Cell viability was assessed microscopically by trypan blue exclusion analysis. Briefly, purified goat anti-human IgA (100 μg), IgE (100 μg) and IgG (100 μg) antibodies (1·5 mg/ml; Medac, Hamburg, Germany) were labelled with 37 MBq of $Na^{125}I$ (specific activity: 520·2 MBq per μg of iodine; Amersham Buchler) as described by Klinman and Taylor.[15]

Removawell U-bottomed wells (Dynatech, Denkendorf) were coated with anti-IgA [100 μl, diluted 1:1000 in phosphate-buffered saline (PBS) supplemented with 0·05% Tween 20] or anti-IgE (100 μl, 1:1000 in PBS, 0·05% Tween 20) or anti-IgG (100 μl, 1:1000 in PBS, 0·05% Tween 20) for 4 hr.

A 100-μl aliquot supernatant (IgA- and IgG-RIA: 1:10–1:20 dilution) was added and incubated overnight at room temperature. [^{125}I]anti-IgA (100,000 c.p.m.) or [^{125}I]anti-IgE (100,000 c.p.m.) or [^{125}I]anti-IgG 100,000 c.p.m.) was added for an additional 4 hr at 37°. In parallel, standard curves for IgA (0·3–300 ng/ml), IgE (0·2–200 ng/ml) and IgG (0·3–300 ng/ml) were performed.

The detection limits for IgE and IgA or IgG were 0·4 ng/ml and 0·8 ng/ml, respectively.

The specificity of the assays was confirmed by adding immunoglobulin of other isotypes to rule out the possibility of cross-reactivity.

All determinations were made at least in triplicate.

Determination of CD23

The detection of CD23 on PBL was performed as previously described[16] on Day 4 of the cell culture. The monoclonal antibody mAb 135 was a kind gift from G. Delespesse (University of Montreal, Canada). Briefly, 1×10^6 cells were incubated with ^{125}I-labelled mAb 135 (50 ng/200 μl, 14·8 kBq) for 1 hr at room temperature. Separation of unbound and bound antibodies was carried out by centrifugation through a 500-μl FCS cushion (500 ***g***, 10 min, 4°) in minitubes (Greiner, Nürtingen, Germany). After removal of unbound radioactivity and washing with PBS (0·05% Tween 20) the cell-bound radioactivity was measured with a gamma-counter (Packard Cobra, Packard-Canberra, Frankfurt, Germany). All determinations were made at least in triplicate. The results were indicated as percentage binding of the total applied ^{125}I-labelled mAb 135 per 1×10^6 cells.

Analysis of data

All experiments were performed three times with different donors. The data were calculated as means ± SD. The significance was evaluated with Student's *t*-test for independent means. $P < 0{\cdot}05$ was considered significant.

RESULTS

Experiments were carried out to analyse the effects of teichoic acid, peptidoglycan and enterotoxin B as well as IL-4 and IL-5 on immunoglobulin synthesis and on CD23 expression in PBL from patients with AD.

Immunoglobulin synthesis

Effects of teichoic acid on IgE, IgA and IgG synthesis

In a first series of experiments we investigated the influence of teichoic acid on IgA, IgE and IgG synthesis in PBL. Figure 1a shows that higher concentrations of teichoic acid induce a slight IgE synthesis in PBL from non-atopic donors. The addition of IL-4 or IL-5 did not affect IgE synthesis in normal donors as compared with the controls. In patients with AD stimulation with 1 μg of teichoic acid induced a slight increase in IgE synthesis. The addition of IL-4 and IL-5 induced a suppression of IgE synthesis by about 60% in these patients. The sponta-

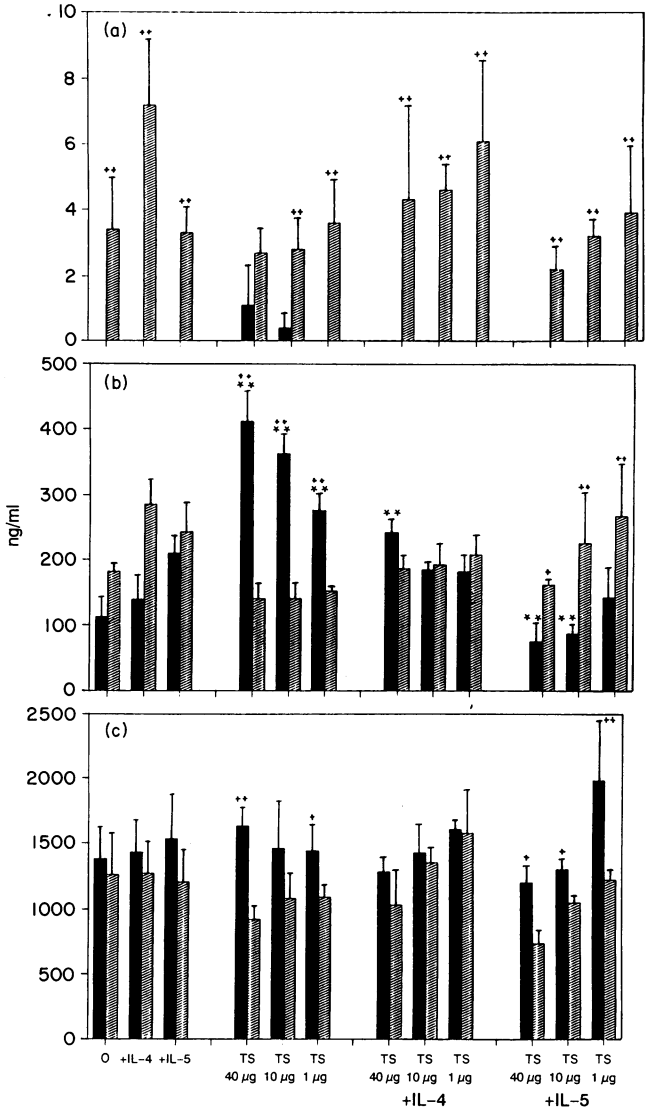

Figure 1. Influence of teichoic acid (40, 10, 1 µg/ml) on spontaneous IgE (a), IgA (b) and IgG (c) synthesis in PBL from normal donors (■) and patients with AD (▨). Means ± SD of three independent experiments in each group. $*P<0.05$ and $**P<0.01$ are significant as compared with spontaneous Ig synthesis + and + +, significant as compared with normal donors.

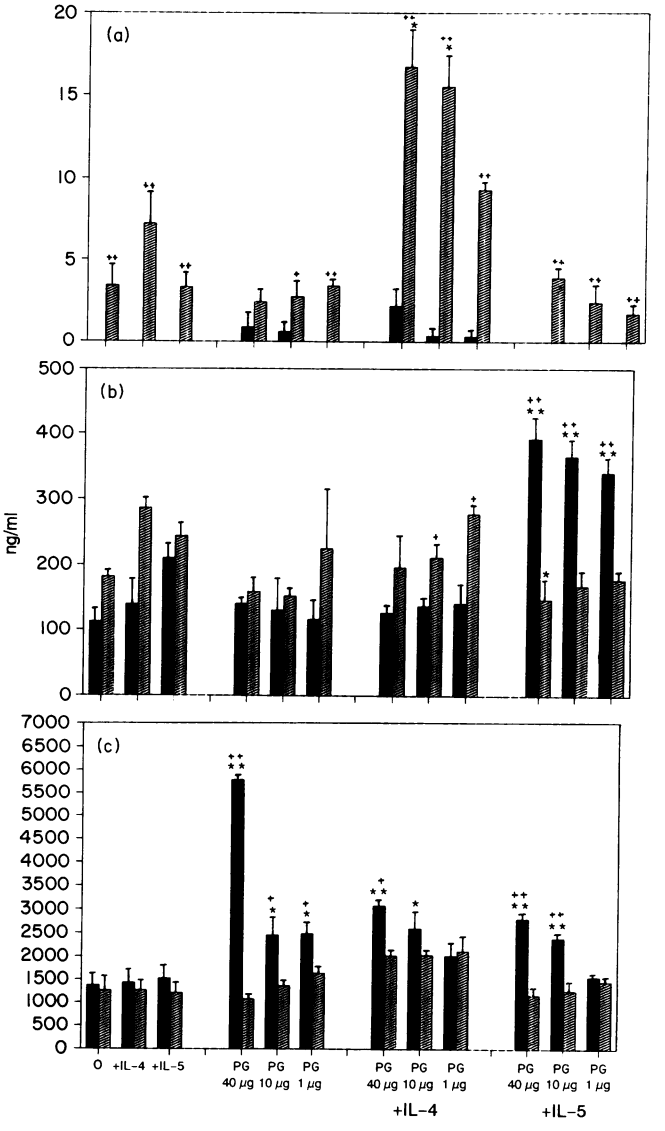

Figure 2. Influence of peptidoglycan (40, 10, 1 µg/ml) on spontaneous IgE (a), IgA (b) and IgG (c) synthesis in PBL from normal donors (■) and patients with AD (▨). Means ± SD of three independent experiments in each group. $*P<0.05$ and $**P<0.01$ are significant as compared with spontaneous Ig-synthesis. + and + +, significant as compared with normal donors.

neous IgA synthesis (Fig. 1b) in normal PBL was stimulated significantly by teichoic acid ($P<0.01$) up to 411 ± 54·7 ng/ml. Co-stimulation with IL-5 and teichoic acid suppressed the IgA synthesis significantly ($P<0.01$) as compared with the control. However, in patients with AD teichoic acid led to a marked suppression of IgA synthesis by about 80% ($P<0.05$). Figure 1c shows that IgG synthesis neither in normal donors nor in patients with AD was affected by teichoic acid alone. Co-stimulation of PBL with teichoic acid and IL-5 induced in AD patients a significant suppression of the IgG synthesis ($P<0.05$).

Role of peptidoglycan in IgE, IgA and IgG synthesis

Figure 2 shows the effects of peptidoglycan on immunoglobulin synthesis. In normal donors peptidoglycan induced a slight IgE synthesis which was enhanced after addition of IL-4. The production of IgE in patients with AD was significantly ($P<0.05$) stimulated by peptidoglycan and IL-4 up to 16·7 ± 2·3 ng/ml (Fig. 2a). IgA synthesis (Fig. 2b) in PBL from normal donors was not stimulated by peptidoglycan alone or combined with IL-4. However, co-stimulation of non-atopic PBL with peptidoglycan and IL-5 led to a significant ($P<0.01$) increase in IgA synthesis up to 391 ± 34·9 ng/ml as compared with the IL-5 control. In contrast to these results 40 µg of peptidoglycan and IL-5 suppressed IgA synthesis significantly ($P<0.05$) in patients with AD.

Figure 2c shows that peptidoglycan induced a very strong stimulation of IgG synthesis in normal donors up to 5779 ± 102 ng/ml ($P<0.01$). This stimulatory effect was not markedly affected by IL-4 and IL-5. IgG synthesis in PBL from patients

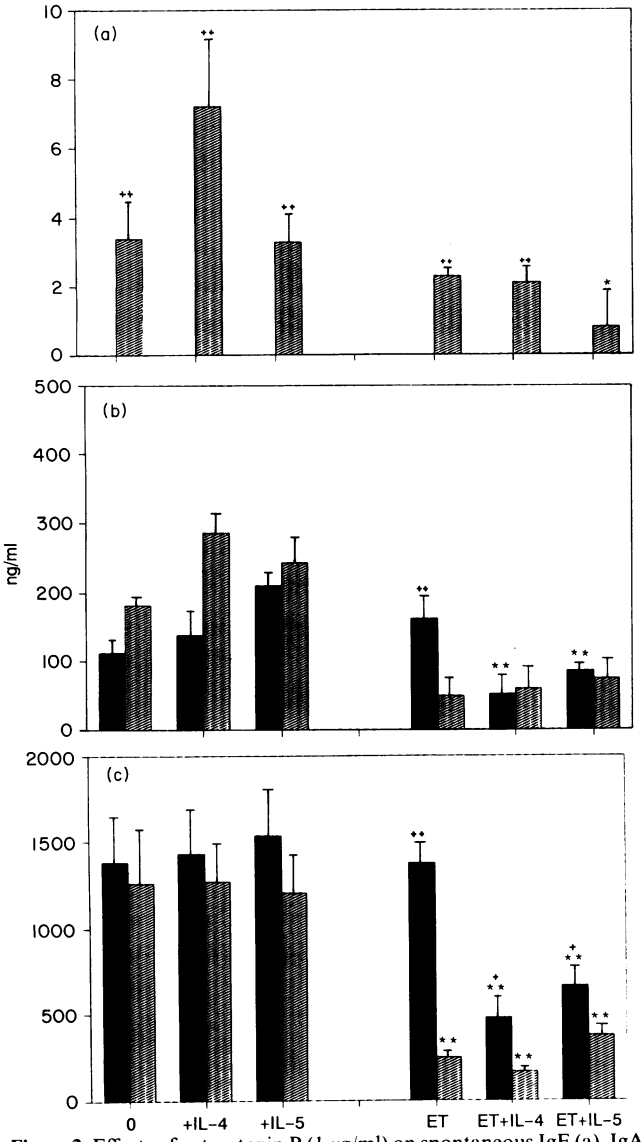

Figure 3. Effects of enterotoxin B (1 μg/ml) on spontaneous IgE (a), IgA (b) and IgG (c) synthesis in PBL from normal donors (■) and patients with AD (▨). Means ± SD of three independent experiments in each group. $*P<0.05$ and $**P<0.01$ are significant as compared with spontaneous Ig-synthesis. + and + +, significant as compared with normal donors.

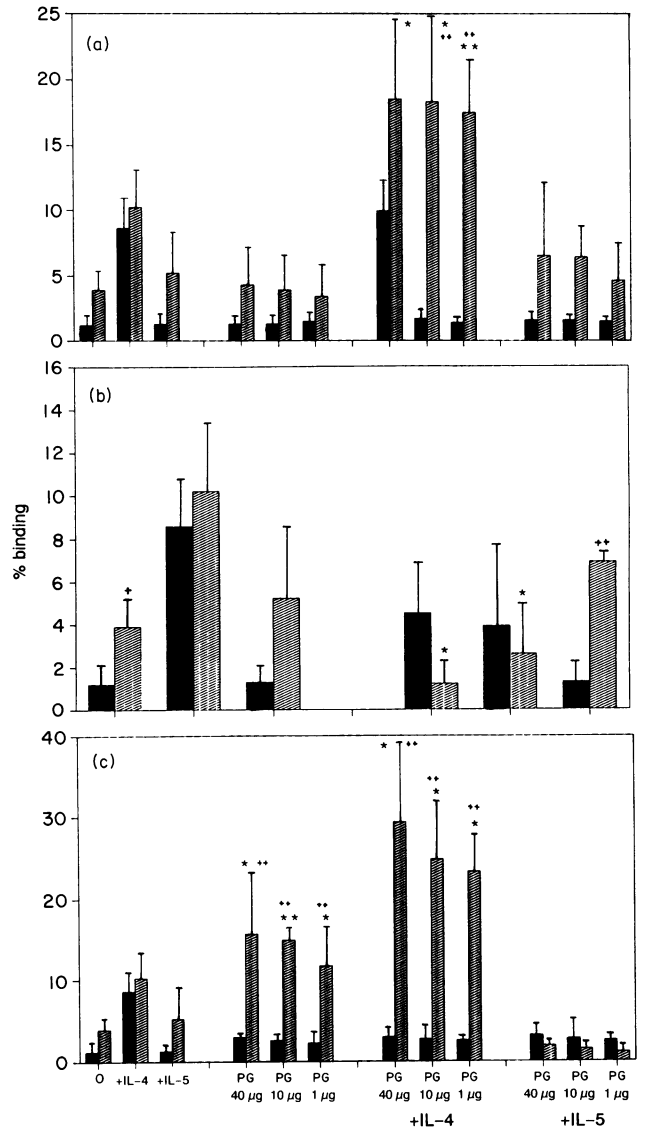

Figure 4. Effects of teichoic acid (a) and peptidoglycan (b) (40, 10, 1 μg/ml), as well as enterotoxin B (c) (1 μg/ml), IL-4 (20 U/ml) and IL-5 (10 U/ml) on CD23 expression. Means ± SD of three independent experiments in normal donors (■) and patients with AD (▨) $*P<0.05$ and $**P<0.01$ are significant as compared with the PBL control. + and + +, significant as compared with normal donors.

with AD was enhanced significantly ($P<0.01$) only by peptidoglycan and IL-4.

Effects of enterotoxin B on IgE, IgA and IgG synthesis

The role of staphylococcal enterotoxin B as a superantigen that strongly modulates T-cell functions has been shown.[17] In subsequent experiments its effect on immunoglobulin regulation was studied.

Figure 3a shows that enterotoxin B had no effect on IgE synthesis in PBL from normal donors, whereas IgE synthesis in patients with AD was significantly ($P<0.05$) suppressed when IL-4 or IL-5 was added. IgA production in non-atopic PBL was not modulated by enterotoxin B alone. However, co-stimulation with IL-4 or IL-5 led to a significant ($P<0.05$) suppression of IgA synthesis (Fig. 3b). IgA synthesis in PBL from patients with AD was suppressed markedly ($P<0.01$) by enterotoxin B alone (48·7 ± 32·4 ng/ml) as compared with spontaneous IgA secretion (181 ± 13·9 ng/ml). The addition of interleukins did not modulate the suppressive effect induced by enterotoxin B. The results obtained were similar when IgG synthesis was studied (Fig.3c).

CD23 expression

In addition to Ig synthesis, modulation of CD23 expression in PBL from normal and atopic donors by teichoic acid, peptidoglycan and enterotoxin B was studied (Fig. 4a–c).

Figure 4a shows the effects of teichoic acid. CD23 expression in normal PBL was not affected, whereas CD23 expression in PBL from patients with AD was significantly enhanced

(18·5 ± 6·9% binding) when IL-4 was added. Higher concentrations of peptidoglycan enhanced the expression of CD23 in PBL from patients with AD. Without IL-4 40 μg of peptidoglycan induced a fivefold higher expression of CD23 as compared with the control ($P < 0.05$). The addition of IL-4 led to an expression of CD23 up to 29·3 ± 9·5% binding (Fig. 4c).

In contrast to these results, enterotoxin B (Fig. 4b) enhanced CD23 expression in normal donors, whereas CD23 expression in PBL from patients with AD was significantly suppressed in the presence or absence of IL-4 ($P < 0.05$).

DISCUSSION

The high incidence of chronic skin colonization with coagulase-positive staphylococci is a prominent feature in AD. Although many attempts have been made to quantify and characterize the micro-organisms on the skin of patients with AD as well as to detect specific IgE antibodies against *S. aureus* in these patients, only few data are available with regard to the host defence mechanisms in this disease. Recently, we demonstrated that heat-killed *S. aureus* suppresses the synthesis of IgA and IgG, but in addition stimulates IgE synthesis and CD23 expression in patients with AD.[7]

The purpose of this study was to analyse whether these effects of whole bacteria are caused by distinct cell wall products or toxins of staphylococci.

The major components of the cell wall of *S. aureus* are peptidoglycan (50%), teichoic acid (40%) and protein A (5%).

The cell wall products of *S. aureus* we investigated, teichoic acid and peptidoglycan, induced, as well as enterotoxin B, a suppression of IgA and IgG synthesis in patients with AD. These effects were less pronounced but still significant with the isolated bacterial substances as compared with the results obtained with intact cells. One may assume an additive effect of teichoic acid, peptidoglycan and enterotoxins on Ig synthesis.

IL-4 is known to be a cytokine that induces IgE synthesis and the expression of the low-affinity receptor for IgE ($Fc_\varepsilon RII$, CD23). Therefore, an important role for it in the pathogenesis of allergic diseases as well as in AD has been suggested.[18,19] IL-5 has also been implicated in the induction of allergic diseases; it enhances IgE synthesis in the presence of IL-4 and leads to eosinophil maturation. In addition, a role in the control of IgA synthesis has been described.[20] Therefore the influence of this interleukin on the observed effects of the isolated bacterial substances was analysed.

In our experiments co-cultures of normal PBL with teichoic acid, peptidoglycan or enterotoxin and with IL-4 as well as with IL-5 significantly modulated IgA synthesis. In normal donors addition of IL-4 and particularly IL-5 led to a suppression of the teichoic acid- and enterotoxin B-induced IgA synthesis similar to the suppression obtained in patients with AD. Only co-stimulation of non-atopic PBL with peptidoglycan and IL-5 enhanced IgA synthesis. It may be suggested that the dysregulation of T-cell activation by these bacterial products and of the subsequent cytokine-dependent Ig synthesis may be responsible for the suppressed IgA synthesis in patients with AD. Interestingly, enterotoxin B and IL-4 as well as IL-5 had similar effects when IgG synthesis was studied.

Enterotoxin B belongs to a group of bacterial exotoxins that strongly and specifically stimulate $CD4^+$ and $CD8^+$ T lymphocytes by cross-linking the T-cell-antigen receptor with major histocompatibility complex class II molecules on accessory or target cells. This mechanism is due to the recognition of a specific antigen by T lymphocytes.[17,21] It has been shown that the majority of the isolated *S. aureus* strains from the skin of patients with AD produce 'superantigens', e.g. enterotoxin B, unlike *S. aureus* strains obtained from the skin of non-atopic donors (Dr D. Leung, personal communication). The reason for this is still unclear; it may be that the disturbed skin environment in patients with AD supports the production of exotoxins by *S. aureus* during chronic colonization. Patients with dermatitis may, via lesions of the skin surface, express dermal fibronectin receptors which increase the adherence of *S. aureus* to the skin.[22] An innate increase in adherence of *S. aureus* via adhesions, e.g. protein A,[23,24] as well as other receptors on lymphocytes, e.g. HLA-DR,[25] may via direct interactions mediate a suppression of Ig synthesis in patients with AD.[26]

The disturbed T-cell function in AD may lead to an altered production of cytokines after stimulation with these 'superantigens'. The different cytokine pattern and the well-known immunosuppressive effects of *S. aureus*-derived exotoxins via cytotoxic T lymphocytes against antigen-presenting cells or B lymphocytes which carry the toxin[21] may be the reason for the suppressed IgA and IgG synthesis in patients with AD.

There is also evidence that in patients with AD an enhanced release of soluble CD23 (sCD23) is correlated with IgA suppression.[27] To what extent the induction of CD23 expression by *S. aureus* and the resulting sCD23 release in AD may interfere with the observed IgA suppression has to be evaluated.

At present, there are various hypotheses as to why there is increased IgE production in patients with AD. In normal PBL, IgE synthesis is induced either in the presence of IL-4, which requires T–B-cell contact, or via a cross-linking of CD40 on B cells by antibodies in the presence of IL-4 without T cells.[28]

Previously, it has been shown that heat-killed *S. aureus* induces IgE synthesis and CD23 expression in patients with AD.[7] In fact, the present results show that peptidoglycan and IL-4 enhance synthesis of IgE and CD23 expression in patients with AD very strongly. Teichoic acid and IL-4 also significantly induced CD23 expression but not IgE synthesis in patients with AD.

It may be speculated that induction of IgE synthesis is mediated via an imbalance of the cytokine pattern[29] after T-cell activation or by a direct interaction of staphylococcal cell wall components with receptors on B lymphocytes.

It has been shown that peptidoglycan teichoic acid and protein A induce histamine release.[30] In patients with allergic asthma *Bordetella pertussis, Haemophilus influenzae* and lipopolysaccharide (LPS) also caused bronchoconstriction and enhanced release of histamine and induce the production of specific IgE antibodies.[31,32] In this regard a correlation between salivary IgA deficiency and atopy has been discussed.[33]

These results indicate that *S. aureus* induces via such bacterial substances the release of inflammatory mediators in patients with AD. Bacterial components may function in a similiar way as aeroallergens which penetrate the skin and support allergic skin reactions in AD via binding to IgE molecules on the surface of Langerhans cells[34] and a subsequent stimulation of T cells to produce IL-4, which induces an increased IgE synthesis.

Summarizing our data, it is not possible to decide conclusively whether the chronic colonization of the skin in AD is an

epiphenomenon or the result of distinct defects in host defence mechanisms. However, the fact that patients with AD have multiple defects in their immune system makes it probable that the depression of IgA and IgG synthesis by *S. aureus* and the influence of IL-4 and IL-5 *in vitro* is the result of an altered immune response against bacterial components in this disease. This defect may then lead to a decreased resistance against *S. aureus* on the skin surface owing to a reduced concentration of immunoglobulins.

The effects on IgE synthesis and CD23 expression may indicate that *S. aureus* supports acute and chronic allergic reactions in patients with AD.

ACKNOWLEDGMENTS

K.N. was supported by Bundesminister für Forschung und Technologie and W.K. was supported by Deutsche Forschungsgemeinschaft and Bundesanstalt für Arbeitsschutz.

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